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Award Number: DAMD17-00-1-0159

TITLE: Screening for Breast Cancer Using Near Field Infrared Spectroscopy of a Single Strand of Hair

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REPORT DATE: August 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 2001	Annual (01 Aug 00 - 31 Jul 01)

4. TITLE AND SUBTITLE
Screening for Breast Cancer Using Near Field Infrared Spectroscopy of a Single Strand of Hair

5. FUNDING NUMBERS
DAMD17-00-1-0159

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

A recent Australian study has used synchrotron x-ray diffraction to identify changes in the structure of hair that may be linked to either the occurrence of breast cancer, or the increased predisposition to breast cancer because of the breast of a mutation of the *BRCA1* gene. We would like to develop a new method for the screening of breast cancer based on infrared spectroscopy of a single strand of human hair. Our study will provide an independent test of the proposed link between hair structure and breast cancer. In addition it may also provide a detailed understanding of how deposits in hair are linked to the formation of breast cancer, at a molecular level.
Synchrotron x-ray studies require the use of large accelerators. Our proposed instrument is a table-top device with the possible potential for rapid, non-invasive, safe and inexpensive screening. This work involves an interdisciplinary collaboration between physicists, epidemiologists, and oncologists at Boston University Center for Photonics, Dartmouth College, and Dartmouth Hitchcock Medical Center.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
10

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

This is an ongoing proposal for performing high resolution infrared microscopy on a single strand of human hair. This work was motivated by synchrotron x-ray scattering experiments that suggested that there are structural changes in hair samples taken from women who either have breast cancer, or have an increased genetic risk of the disease. Because infrared spectroscopy is uniquely capable of characterizing and identifying biomolecules from their spectral "fingerprints", this work has the potential for:

(i) providing an independent test of the x-ray diffraction studies, and providing insight into the molecular basis for the structure and composition of human hair linked to the formation of breast cancer;

(ii) leading to the development of a novel method of screening for early breast cancer based on a table-top high-resolution vibrational infrared microspectrometer.

This report describes the progress made in the first year of this project, and discusses the relationship between the infrared and x-ray work. This project involves an interdisciplinary collaboration between physicists, epidemiologists, and oncologists at Boston University Center for Photonics, Dartmouth College, and Dartmouth Hitchcock Medical Center. Consultations with collaborators has resulted in an important new aspect of the proposed studies, where the infrared work is tied closely to x-ray diffraction work.

Update on x-ray diffraction studies in the literature. Since the submission of the original proposal there have been additional developments on the x-ray diffraction studies that impact on this work. So we have taken the liberty of summarizing some of the recent work below.

We summarize (very briefly) the state of the x-ray work so far.

1) James and her collaborators first report the correlation between a certain diffuse ring in the small angle x-ray scattering using synchrotron radiation, and the incidence of breast cancer [James et al, 1999].

2) Several separate groups publish criticisms (Meyer et al, 2000; Howell et al, 2000; Briki et al, 1999), showing data that appear to contradict the original James et al result.

3) Meyer and James (2001) report a rebuttal, affirming the essential findings of the original James et al paper, and arguing the need for following a consistent protocol in acquiring x-ray data.

The x-ray indicates that while there continues to be some disagreement about the initial findings of structural changes in hair linked to breast cancer, there may be some hint of convergence, because one of the authors, P. Meyer, has published on both sides of the question! There is general agreement that the diffraction ring identified by James et al (1999) does occur in hair samples, and is related to lipid deposits. The issue is whether these rings are linked to breast cancer. The relationship with lipids suggests that the original premise of our proposal – namely, that infrared microspectroscopy can offer an independent tool for studying the same structural changes – is now on very firm ground.

Key Research Accomplishments

Summary: This project consists broadly of two distinct phases. The first phase is an instrumentation phase, where the feasibility of obtaining high-resolution infrared spectra from a single strand of human hair was to be demonstrated. This task has been essentially accomplished. The second phase, to start in the second year of the proposal, proposed to study hair samples from control subjects and cancer patients with a view to testing the x-ray diffraction studies. The second stage is well under way, and we have some initial progress to report in this stage also. Although the statistical samples collected in the first year are too few in number for us to make definitive statements, preliminary studies have been very promising. These studies, along with new papers that have been published on x-ray diffraction, have led to the suggestion that we should perform simultaneous x-ray and infrared studies on the same samples of hair. Such a "coregistration" of infrared and x-ray work was not envisioned in the original proposal. We are pleased to report that one of the best groups in the world in doing small angle X-ray scattering, headed by Prof. Sol Gruner at Cornell University, has agreed to perform x-ray scattering studies on the same hair samples collected at Dartmouth Medical College. Prof. Gruner is the Director of CHESS, (synchrotron at Cornell) and his group is recognized as being one of the best groups in the world at using both conventional and synchrotron x-ray sources for Small Angle-X-ray diffraction.

We anticipate that all the objectives of the proposal will be met by the time the proposal concludes.

At the present time, therefore, while the x-ray work remains extremely promising, there is urgent need for independent verification from other techniques. Given the conflicting nature of published x-ray work, we decided to extend our infrared work to perform concurrent x-ray diffraction work. This additional work will be done in collaboration with Prof Sol Gruner's group at Cornell University, at no additional cost to this project. We feel that such concurrent

studies infrared and x-ray studies on samples from the same set of patients are essential in order to test the x-ray work and to characterize the underlying biomolecular changes in the hair that may be associated with the disease.

In the next section, we describe briefly (i) the results of the initial infrared microscopy work, showing that it is possible to obtain high resolution infrared spectra from a single strand of human hair; (ii) comparison of infrared spectra corresponding to protein and lipid vibrational modes ; (iii) a brief description of preliminary studies on hair from a number of patients; (iv) motivation for combining infrared and x-ray work.

Reportable Outcomes

Vibrational Infrared Microscopy of a single strand of human hair: In the first 6 months of the project, sections of human hair were taken using a cryomicrotome, and transferred to an infrared transmitting calcium fluoride window. Fig 1 shows optical images combined with infrared spectroscopy on the same strand of hair, in both longitudinal and transverse sections. Both sections were analyzed because the x-ray diffraction studies suggested that lipid deposits that may be responsible for observed x-ray patterns may be randomly oriented.

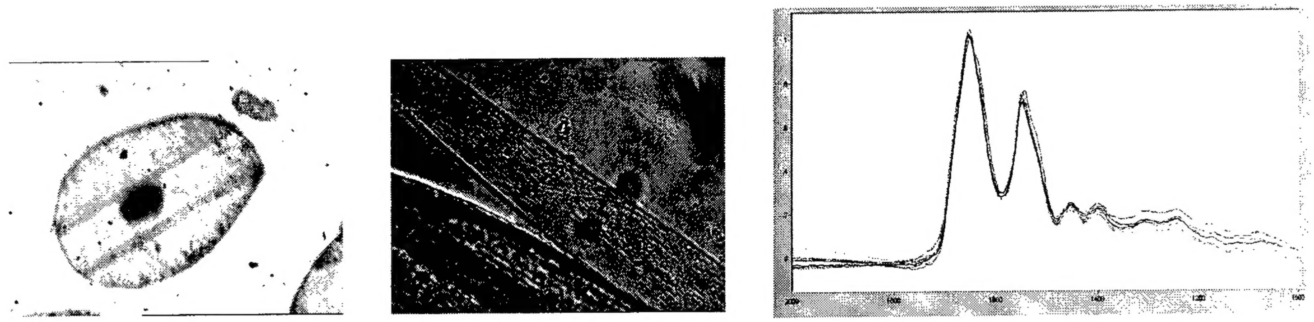

Figure 1: (a) Cross section of single strand of human hair; (b) Longitudinal section of hair; (c) Infrared spectra obtained from the hair sample, showing the primary amide I and amide II bands (C. M. Smith).

One question that concerns infrared studies was whether routine hair treatment, such as shampooing has a significant effect on the infrared spectrum. Control subjects were requested to collect hair samples before and after shampooing, and then processed Hair samples were collected from control subjects. After collecting coarse resolution infrared spectra, an infrared map was generated, where spectra are collected from different locations.

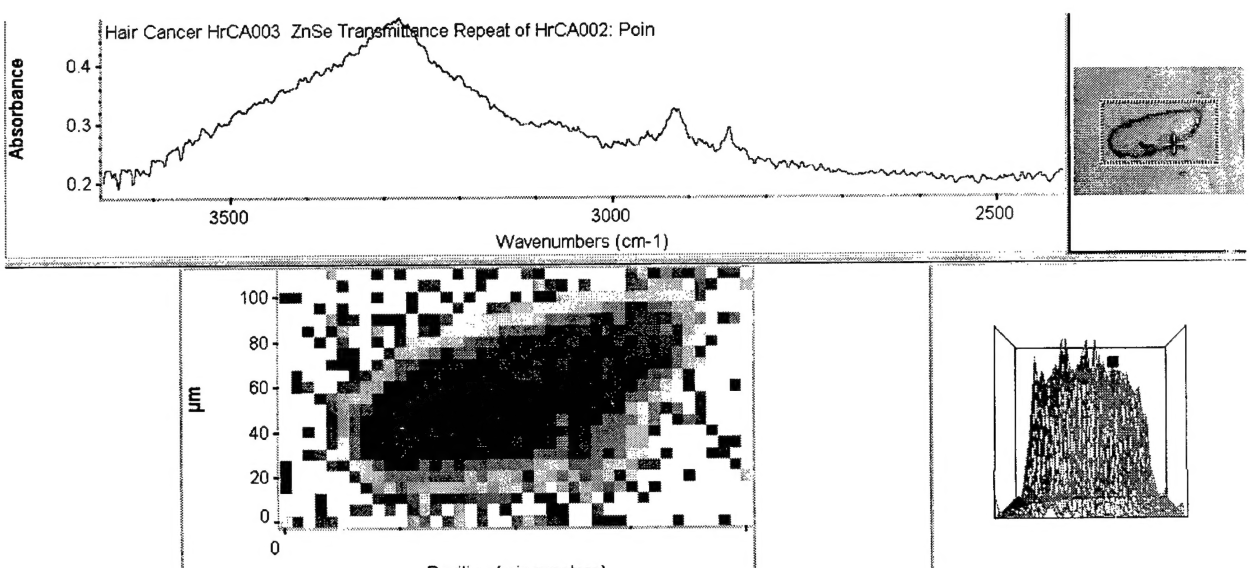

Figure 2. Infrared spectrum from one specific point (shown in the inset figure, top right), in the 3000 cm^{-1} vibrational region. The small peaks are mostly due to lipid stretching vibrations. These features show significant variation from site to site in the hair sample.

High resolution infrared studies were performed on cryomicrotomed sections of hair, placed on Zinc Selenide windows. Spectra were collected from several thousand points within a hair sample, with attention being paid to the protein amide regions, as well the CH stretch regions near 3 microns, where lipids are known to contribute significantly.

Samples from five patients were collected at Dartmouth College Medical Center, in accordance with approved IRB protocols. Infrared data were compared to the spectra obtained from control samples, obtained at Boston. Variations were observed in the lipid absorption bands from one subject to another. Variations were also observed from one site to another within a single hair sample, suggesting that lipid distribution was not uniform across the hair.

At this point, after discussing the data with Dr. John Baron, M.D., at Dartmouth Medical Center, it became clear that it would be very useful if we could have performed x-ray studies on the same hair fibers that were used for infrared work. This would allow a possible "coregistration" of infrared images and regions that contribute to the x-ray signal.

Samples have now been sent to Prof Gruner's group at Cornell, and we anticipate that x-ray data will become available in time for the next report.

Personnel: Hair samples from patients were obtained by Dr. Peter Kaufman, M.D., Dartmouth Medical College. Initial studies reported were performed by C.M. Smith, under the supervision of the Principal Investigator and Prof. M. K. Hong at Boston University. We are pleased to say that Mr. Smith has now enrolled in Medical School, and we trust that the work on this project was responsible in part in motivating him. We also have another medical student, Ji-Yeon Kim, working in the laboratory at the Center for Photonics. X-ray work at Cornell is being performed by Dr. Peter Abbamonte, working with Prof. Sol Gruner (at no cost to this project).

Conclusions

We have established that high resolution infrared microscopy is capable of acquiring infrared spectra from both lipid and protein molecules from a single strand of human hair. This justifies extending the experiments to the next phase of the research, where additional spectra will be collected from a statistically significant sample of patients, in our continuing collaboration with physicians at Dartmouth College's Hitchcock Lahey Medical Center. We also plan to extend the studies to perform x-ray diffraction studies, in collaboration with scientists at Cornell University.

The preliminary studies have shown that (i) there are some differences in the infrared spectrum from one subject to another, both in some of the protein bands as well the lipid vibrational bands; (ii) there are significant variation in the lipid absorption bands from site to site *within* a hair sample from a single patient.

Two questions arise immediately, triggered by the above two observations: (i) Are the changes in vibrational spectra we observe related in any way to structural changes in hair, of the kind observed b y synchrotron x-ray diffraction? (ii) Are the spatial variations in the lipid absorption bands within a single hair sample due to the presence of lipid deposits (again as indicated by x-ray studies).

At present the statistics are too small to make a clear statement about either one of these questions. All we can say is that the infrared studies remain extremely promising.

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